THE INTERACTION OF TWO FRAGMENTS OF PULSATING HEART TISSUE.

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PLATE 28.

(Received for publication, September 27, 1923.)

It has seemed possible that a synchronous contraction could be obtained *in vitro* of two different, separated fragments of heart, if anastomosis between the autonomic contractile elements could be established. In the present paper experiments are reported on the theme.

Technique.

First the optimal conditions were determined under which fragments of embryonic heart tissue from chicken continue to pulsate *in vitro*. The best results were obtained when the plasma was diluted with Ringer or Tyrode solution to about 20 per cent. One volume of a 20 per cent plasma in Ringer solution and one volume of fresh embryonic tissue juice were the composition of the culture medium.

The fragments of heart were obtained from chicken embryos approximately 8 to 10 days old. In some of the experiments the fragments came from different embryos, and in some they were derived from the same heart, while in yet others two fragments derived from the same heart had their cut edges brought into close approximation. This was accomplished in the following way. A rather large fragment of heart was transferred to the culture medium and while the plasma and embryonic juice were still in a liquid state, the fragment was divided with a clean cut and the two pieces kept apposed by means of a preparation needle and the point of a cataract knife until coagulation of the plasma had taken place.

Usually three fragments were placed in one culture, two adjacent to each other and one separately as a control.

A couple of hours after the preparation of the cultures, the rate of contractions of the various fragments was recorded. The interval of time between two contractions was checked for the control (the isolated fragment) and for each of the two adjacent fragments, by means of a stop-clock. The observations were repeated on the 3 to 4 days following, after which the heart fragments ceased to pulsate.

The distances between the two adjacent fragments in the culture were purposely varied from direct contact to about the width of one of the fragments, *i.e.* about 1 mm.

Results.

When several fragments of heart tissue were embedded in the same culture medium, no two of the pieces pulsated at the same rate. The intermission between successive contractions was the same for any given piece but not like that for others.

In experiments in which the distance between two adjacent fragments was about 1 mm., it was noticed that the intermission between two contractions remained constant over a long period of time. This rhythmic contraction for the individual fragments lasted about 20 hours by which time the outgrowth of new cells, mainly fibroblasts, had bridged the zone between the two pieces (Fig. 1). From now on the pulsation became irregular. Long intermissions were followed by periods of quick contraction or of fibrillar contractions (Table I). Meanwhile the isolated fragment in the same culture which served as a control had continued to pulsate with a constant time interval. The culture was now unsealed and by means of a clean cut with a sharp cataract knife the two adjacent bits were separated in the zone of new growth. The culture was resealed and again observed under the microscope. The procedure of separation lasted only about 1 minute. From now on, the contractions of the two pieces were rhythmic, *i.e.* the intermission between the contractions was constant for each of the two pieces but not synchronous (Table I).

The interpretation of this phenomenon is difficult. It may perhaps be explained by a mechanical interference with contraction after the fragments have grown together.

When the two fragments of heart tissue had been placed very close to each other in the culture they pulsated as one, that is to

TABLE I.

Culture 2013.

Time between two contractions of control.	Time between two contractions of one of two adjacent fragments.	Time between two contractions of the other.
Before the two became connected.		
	sec.	*sec.*
	2.0	1.6
	2.0	1.6
	2.0	1.6
	2.0	1.4
	2.0	1.6
After the connection.		
sec.		
3.0 3.0	1.5 2.0	1.2 1.0
3.0 3.0	2.0 2.5	20.0 0.8
3.0 3.0	2.0 2.0	1.0 0.6
3.0 3.0	2.0 1.8	0.8 0.8
3.4 3.0	2.1 1.5	1.0 0.8
3.0 3.0	1.5 2.0	35.0 1.0
3.0 3.0	1.5 2.0	1.0 0.6
3.0 3.0	1.5 1.5	0.8 0.4
3.0 3.4	1.5 1.5	0.6 0.6
3.0 3.0	1.5 1.8	1.0 0.8
3.0 3.0	1.8 1.8	0.8 0.4
	1.5 1.5	18.0 0.4
	1.8	1.0 1.0
After separation.		
	5.0 5.0	8.0 8.0
	5.0 5.0	8.0 8.0
	5.0 5.0	8.0 8.0
	5.0 5.0	8.0 7.0
	5.0 5.0	7.0 7.0
	5.0 5.0	7.0 7.0
	5.0 5.0	7.0 7.0
	5.0 5.0	7.0 7.0
	5.0 5.0	7.0 7.0
	5.0 5.0	7.0 7.0
	5.0 5.0	8.0 7.0
	5.0 4.0	8.0 8.0

Greatest difference before separation, 1.0.....34.6
Average difference before separation, 0.2.....5.9

Greatest difference after separation, 1.0.....1.0
Average difference after separation, 0.0.....0.1

say synchronously, after about 48 to 72 hours incubation at 39°C. The space between them could scarcely be made out after 48 hours in instances in which the fragments pulsated absolutely synchro-

TABLE II.

Experiment 2040.

Time.	Time between two contractions of one of two adjacent fragments.	Time between two contractions of the other.
hrs.	*sec.*	*sec.*
0	1.6	2.4
0	1.6	2.4
0	1.6	2.0
0	1.6	2.0
0	1.8	2.0
0	1.4	2.0
0	1.4	2.0
0	1.4	2.0
24	1.2	3.4
24	1.2	3.6
24	1.4	3.8
24	1.8	4.0
24	3.0	2.4
24	2.2	2.2
24	4.6	2.0
24	4.2	1.6
	Interval between the synchronous contractions.	
48		
48	3.0	
48	3.2	
48	3.0	
48	3.4	
48	3.6	
48	4.2	
48	2.6	
48	3.0	
48	3.0	

nously. The data of such an experiment are given in Table II. The synchronism in this case was first obtained after 48 hours. The experiment also typifies the results with fragments of heart derived from two different chick embryos.

A rather high percentage of success in obtaining synchronous pulsation of fragments of heart resulted when two cut edges were brought directly in contact with each other and especially when those resulting from the same cut were apposed. For the latter purpose a piece of heart was cut in two in culture medium already prepared

TABLE III.

Experiment 2066.

Time.	Time between two contractions of one of two adjacent fragments.	Time between two contractions of the other.
hrs.	*sec.*	*sec.*
0	1.2	4.0
0	1.2	3.8
0	1.2	4.0
0	1.2	4.0
0	1.2	4.0
0	1.0	4.0
0	1.0	4.0
0	1.0	4.0
Interval between the synchronous pulsations.		
24		
24	4.0	
24	0.2	
24	0.1	
24	0.1	
24	0.1	
24	0.1	
24	0.1	
24	0.1	
24	4.2	
24	4.0	
24	0.1	
24	0.1	
24	0.1	
24	0.1	

and the pieces kept in contact until coagulation took place. By this method a synchronous pulsation could be established within 24 hours (Table III).

A good many experiments were made to see if it was possible to obtain synchronous contractions between two fragments of heart,

one from a duck embryo and the other from a chicken embryo. Duck embryo tissue from the heart can be successfully cultivated *in vitro* in a heterologous culture medium composed of chicken plasma and chick embryo juice. The growth was just as extensive as that of the chicken tissue. Fragments of heart from duck embryo were able to pulsate vigorously through several passages in the chicken culture medium. It was possible, therefore, to determine whether tissues of the same organ derived from different species were able to unite and become a physiological unit. The result of these experiments was that no synchronous contractions could be obtained between fragments of heart, one of them from duck and the other from chicken embryo.

Serial sections were made of the cultures. A definite histologic difference could be observed in the zone between fragments that had pulsated synchronously and others that had not. In the line of contact of the latter, the contractile elements were separated by a layer of connective tissue (Fig. 1), whereas in the case of the fragments contracting together, the contractile elements from one had established a contact with those of the other (Fig. 2).

DISCUSSION AND SUMMARY.

Fragments of heart from the same species, when cultivated *in vitro*, are capable of uniting and pulsating synchronously. This happens not only in fragments derived from the same heart but with those from different individuals of the same species. It was not possible, on the other hand, to obtain, under the conditions of experiment, any physiological union of a heart fragment from a duck embryo with one from a chicken embryo, though both pulsated in the same medium and though the tissue cells of the duck were capable of growth and multiplication.

From the experiments it is evident that cell contact is essential for the development of physiological identity as just described. Since such a physiological identity cannot be obtained through the union of a fragment of a duck heart and a fragment of a chicken heart, one may suppose that anastomosis between the cells must play a very significant rôle in it.

CONCLUSIONS.

1. It is possible to establish a physiological union *in vitro* of two fragments of pulsating heart from the same species, such that a synchronous pulsation of them is obtained.

2. It has proved impossible to obtain synchronous pulsation in two fragments of heart belonging to different species (duck and chicken).

3. The facts indicate that cellular anastomosis between the contractile elements of the fragments originally separate are the cause for the physiological union of the two.

EXPLANATION OF PLATE 28.

FIG. 1. Experiment 2067. A section through the line of contact of two fragments of chick embryo heart which failed to pulsate synchronously *in vitro*. A layer of connective tissue can be seen separating the contractile elements. × 175.

FIG. 2. Experiment 2066. A section through the line of contact of two fragments of chick embryo heart which had pulsated perfectly synchronously *in vitro*. The contractile elements of the one are in direct contact with those of the other. × 175.

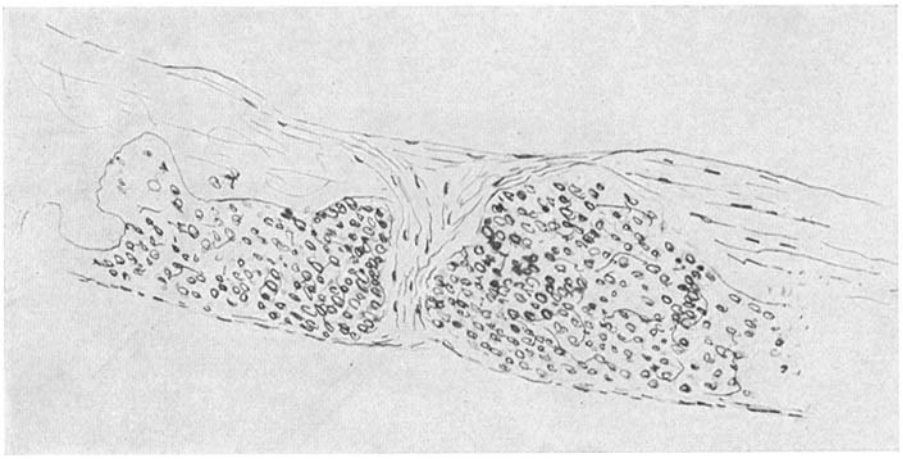

FIG. 1.

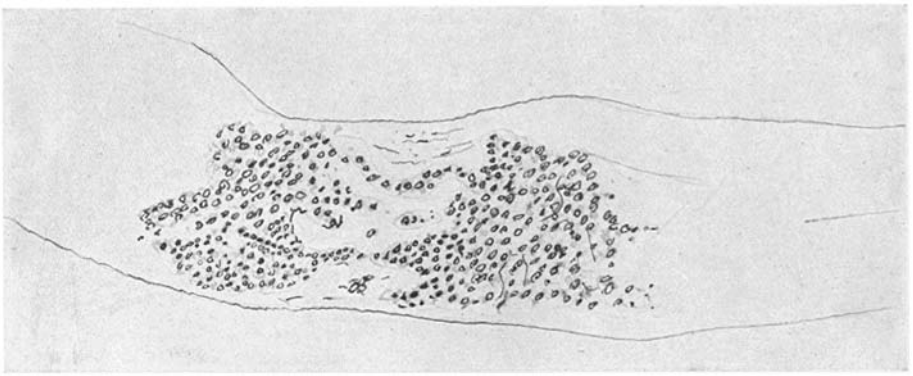

FIG. 2.

(Fischer: Interaction of pulsating heart tissue.)